United States Department of Agriculture
Animal and Plant Health Inspection Service
Center for Veterinary Biologics
P. O. Box 844
Ames, IA 50010

1. Reagent Name: *Clostridium tetani* Toxin

2. Strain or Source: Not applicable

3. Lot Number: IRP 594

4. Fill Date: September 3, 2010

5. Expiration Date: No expiration date has been assigned to this product because tetanus toxin has demonstrated over time to be very stable if properly stored. The stability of this reagent will be routinely monitored by the Center for Veterinary Biologics.

Precautions: Accidental parenteral inoculation and ingestion of the toxin are the primary hazards associated with this reagent. It is uncertain if tetanus toxin can be absorbed through mucous membranes; consequently, the hazards associated with aerosols and droplets remain unclear. The administration of an adult diphtheria-tetanus toxoid at 10-year intervals reduces the risk of toxin exposures to laboratory personnel and is highly recommended.

6. Intended Use: Use IRP 594 to coat microtiter plates for the indirect enzyme-linked immunosorbent assay (ELISA) as described in **SAM 217**, and for the comparative toxin-antitoxin neutralization test in guinea pigs as described in **SAM 206**.

7. Instructions for Use: To conduct ELISA coat each well of the microtiter plate with 100 µL of IRP 594 diluted 1:10 in antigen coating buffer. To conduct comparative toxin-antitoxin neutralization tests in guinea pigs at the 0.10 Antitoxin Unit per mL level, dilute IRP 594 1:52 in 1/15 M phosphate buffered saline, pH 7.4, with 0.2% gelatin (PBS w/gelatin).

8. Test of Reagent: *Determination of the test dose of toxin* – Checkerboard titrations were performed to determine the optimum toxin concentration for adsorption to 96-well microtiter plates. The optimum toxin concentration for the comparative toxin-antitoxin test in guinea pigs was determined by preparing various dilutions of toxin and mixing them with 0.10 American Unit of tetanus antitoxin. The toxin dilution that caused tetanic paralysis in guinea pigs within 60-75 hours, when administered according to 9 CFR 113.451, was selected as the toxin test dose.

Sterility Test - The toxin was tested for sterility and found to be free of viable bacteria and fungi according to 9 CFR, Part 113.26.

9. Container Size, Type, Weight, or Volume: 5.0-mL screw-cap vials containing 2.2 mL of toxin.

10. Storage Conditions: Store IRP 594 at -70°C or lower.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6140 or FAX (515) 337-7673.

12. Origin and Passage History: *Clostridium tetani* culture 7010 obtained from Burns Biotech was used to produce IRP 594. The history of the culture prior to being sent to the Center for Veterinary Biologics is unknown.

13. Method of Preparation: The culture was grown in a 14-liter fermentor vessel containing modified Mueller and Miller medium. The culture was incubated at 35°- 36°C for at least 110 hours. The culture was centrifuged at 10,000 x g in a rotor chamber temperature of 2º- 7°C for 45 minutes. The supernatant was passed through a 0.5-µm Pall Preflow™ DCF filter, then through a 0.2-µm Pall Supor® DCF filter. The filtrate was concentrated using a Millipore pellicon cassette system containing a 10,000 NMWL PTGC00005 filter. The material retained by the filter was passed through a sterile Millipore filtration system containing a 0.22-µm membrane.

14. Other: None

Reagent orders and feedback should be sent *including phone number* to the following email address: CVB@aphis.usda.gov

Reagent orders forms (APHIS 2018) are available from:
https://www.aphis.usda.gov/library/forms/pdf/APHIS_2018.pdf

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